3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Anti-phospho-Tau (pThr^{205})
Developed in Rabbit, Affinity Isolated Antibody

Catalog Number **T 6694**

Product Description

Anti-phospho-Tau (pThr^{205}) is developed in rabbit using a synthetic phosphopeptide derived from the region of human tau that contains threonine 205 as immunogen. The serum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity towards a non-phosphorylated tau.

Anti-phospho-Tau (pThr^{205}) recognizes human tau (pThr^{205}) (45-68 kDa). Mouse and rat tau (100% homologous) have not been tested, but are expected to react. It has been used in immunoblotting and immunohistochemistry applications.

Tau is a microtubule-associated phosphoprotein (MAP) localized in neuronal axons. It promotes tubulin polymerization and stabilizes microtubules.[1] The biological activity of tau is regulated by its degree of phosphorylation.[1,2] Hyperphosphorylated tau is the major protein of the paired helical filaments (PHFs), which make up the pathological neurofibrillary tangles of Alzheimer's disease (AD). The PHFs are also found in the lesions of other central nervous system disorders.[3,4]

Tau phosphorylation involves numerous kinases: glycogen synthase kinase 3β (GSK-3β), MARK kinase, MAP kinase, protein kinase A and C, cyclin-dependent kinase 5 (Cdk5), p38 kinase, c-Jun N-terminal kinase, and casein kinase II.[1,2,5,6,7] Combined tau protein kinase II (TPKII), which consists of Cdk5 and GSK-3β, is the most potent phosphorylation agent indirectly involved in the regulation of the phosphorylation state of tau in neuronal cells.[6,8] In addition, tau is phosphorylated *in vitro* by osmotic cellular stress, which activates the stress-activated protein kinases (SAPKs).

To date, a total of 25 abnormal phosphorylation sites have been identified on hyperphosphorylated tau in AD brain.[10] Normal tau has approximately eight phosphorylation sites. The abnormal phosphorylation occurs usually on serine and threonine residues. Specifically, TPKII phosphorylates serines 202 and 404. GSK-3β transfection phosphorylates serines 199, 202, 235, 396, 404 and 413, and threonines 205 and 231. These sites are among the major abnormal phosphorylation sites of tau.[11] Phosphorylation on these sites reduces the ability of a given tau species to promote microtubule self-assembly.[11,12]. Okadaic acid increases phosphorylation at threonine 231 and serines 235, 396 and 404. Phosphorylated serine 422 was found in the biopsies of brains from patients with Down syndrome, amyotropic lateral sclerosis, corticobasal degeneration, and Pick's disease. It was absent from control group of normal brains.[13]

The opposite process, tau dephosphorylation, is controlled by different protein phosphatases expressed in neurons. Protein phosphatases PP2A and PP2B efficiently dephosphorylate tau *in vitro* and restore biological activity in the assembly of microtubules.[3,10,14]

Recently it was discovered that propyl isomerase (Pin1) interacts with tau hyperphosphorylated on threonine 231 and restores the ability of tau to bind to microtubules.

Reagent

The antibody is supplied in 100 µl of Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

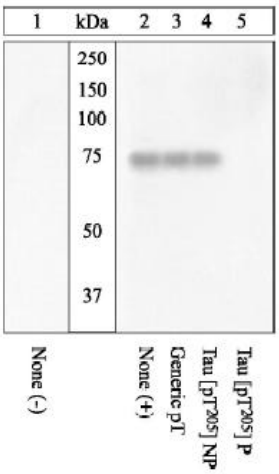
Store at –20 °C. For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, and storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile
A recommended working dilution of 1:1000 is determined by immunoblotting using cell extracts from African green monkey kidney (CV-1) cells, stably expressing human four repeat tau.
Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Peptide Competition

1. NIH3T3 cell extracts spiked with human recombinant tau left untreated (1) or treated with GSK-3β to become phosphorylated (2-5) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer one hour at room temperature.
3. After blocking, membranes were preincubated with different peptides as follow:
   - Lane 1, 2 no peptide
   - Lane 3 a generic phosphothreonine containing peptide
   - Lane 4 non phosphorylated peptide corresponding to the immunogen
   - Lane 5 immunogen
4. After preincubation membranes were incubated with Anti-phospho-Tau (pThr^{205}) antibody for two hours at room temperature in a 1% BSA-TBST buffer.

After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG-HRP and and bands were detected using the Pierce SuperSignal® method.



The data show that only the peptide corresponding to Tau (pThr^{205}) blocks the antibody signal, thereby demonstrating the specificity of the antibody.

References

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